MUTABLE LOCI IN MAIZE

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For the past few years, investigations have been focused on the origin and behavior of mutable loci in maize. The experimental procedures and results have been described in previous reports. In Year Book No. 48 an interpretation of the

mechanism associated with the origin and behavior of the *Ac*-controlled mutable loci was presented. During the past year, emphasis has been placed on expanding this evidence, in order to determine whether or not the premises of the interpretation would continue to be supported by further tests. The results of the year's experiments have substantiated the main conclusions given in the previous report, have provided more information with regard to the mechanisms involved, and have suggested the probable nature of the chromosome materials concerned.

Variegation has been described in a wide range of organisms. Some cases of it are known to be associated with the irregular mitotic behavior of ring-shaped chromosomes, or with chromatin deletion produced by the breakage-fusion-bridge cycle. Another class of somatically expressed variegation is known to be related to irreversible changes in self-reproducing cytoplasmic elements, such as the plastids. A third class of somatic variegation is associated with detectable changes in genic action. The terms mosaicism, mutable genes, somatic variegation, mutable loci, and position effect have been used to designate this class. It is on this third type of variegation that the investigations in maize have been focused. It has been described in many organisms. Because the general nature of the phenotypically expressed instability is so similar in these unrelated organisms, it is difficult to avoid the conclusion that a common principle of nuclear and chromosomal organization and functioning is concerned. The evidence obtained during the past year has made it possible to formulate a working hypothesis that incorporates such a principle. The hypothesis is an expansion of the interpretation of the origin and behavior of mutable loci presented in Year Book No. 48. Its salient features may be reviewed here:

A normal, wild-type locus may be totally or partially inhibited in action by the insertion of a foreign piece of chromatin adjacent to it. Total or partial release from inhibition will occur when this foreign chromatin is removed or altered in organization. The insertion, removal, or change in organization of the foreign chromatin occurs because this chromatin becomes adhesive in certain somatic cells at very precise times in the development of a tissue. The adhesiveness causes a rupturing of the chromosome at the adhered positions during the subsequent mitotic cycle, which results in removal, transposition, or alteration in constitution of the chromatin materials involved. The chromatin primarily concerned in these events is heterochromatin. Its behavior as revealed in this study of the origin and expression of mutable loci may reflect one aspect of its normal behavior in the development of an organism.

Mode of Detection of Transpositions of *Ds*

The first direct evidence with regard to the mode of origin and the operation of mutable loci was obtained from study of the *Ac*-controlled mutable c^{m-1} locus. The origin of this mutable locus by a transposition of *Ds* has been reviewed in previous reports. Because transposition of minute bits of chromatin from one location to another in the chromosome complement is basic to the concept of the origin and behavior of mutable loci, extensive investigations of this phenomenon have been undertaken during the past year. Twenty cases of transposition of *Ds* from its standard location in the short arm of chromosome 9 to another position within this arm have been studied. Several cases

of transposition from the new position to still another position have also been investigated. Transpositions of *Ds* from one location to another within the short arm of chromosome 9 were selected for study because the design of the experiments makes it possible to detect such transpositions shortly after they occur, and also because it is possible to locate, readily and accurately, the new positions of *Ds* activity.

An example may be given of the methods used in detecting and locating new positions of *Ds*. Pollen of plants that have one or more *Ac* loci, and carry *I, Sh, Bz, Wx,* and *Ds*-standard in one chromosome 9, is placed on silks of tester plants carrying *C sh bz wx* but having no *Ds* or *Ac* loci. The state of the *Ds* locus in these plants is selected for a high frequency of dicentric-chromatid formation as a consequence of events occurring at *Ds*. On the resulting ear, kernels that receive an *Ac* locus and a chromosome 9 carrying the markers *I, Sh, Bz, Wx,* and *Ds*-standard should show sectors of tissue with the *C sh bz wx* phenotype as a consequence of dicentric- and acentric-chromatid-forming events occurring at the *Ds*-standard location, which result in elimination from the nuclei of the acentric segment of chromatin carrying the markers *I, Sh, Bz,* and *Wx* (for details, see previous reports). With very few exceptions, the expected phenotypic characters are present in the sectors of the kernels carrying the stated markers. The few exceptional kernels that have sectors showing unexpected phenotypic characters are important, for from them are derived the strains having new positions of *Ds* activity.

The new position is often immediately revealed in an exceptional kernel. As an illustration, we may describe a kernel having *Ds* transposed from its standard location to a position between *I* and *Sh*. During the development of such a kernel, a dicentric-forming event, involving sister chromatids at this position, will produce an acentric fragment that carries the two *I* loci and a dicentric chromatid that includes the two sister proximal segments of the short arm, each carrying *Sh, Bz,* and *Wx*. In the dicentric chromatid, the *Sh* and *Bz* loci are physically close to the position of sister-chromatid union. A bridge configuration will be formed by the dicentric chromatid in the subsequent anaphase. Breakage of this bridge can occur at a position anywhere between the two centromeres. The break is most often nonmedian. Consequently, the broken chromatid that usually enters one telophase nucleus carries two *Sh* and *Bz* loci and one *Wx* locus. The sister nucleus receives a broken chromatid carrying only the *Wx* locus. From each nucleus a sector of tissue will subsequently be produced. When the kernel is mature, twin sectors arising from these two sister nuclei will be evident. One sector will show a *C sh bz* phenotype, within which subsectors of *Wx* and *wx* phenotypes will appear. It will be derived from the nucleus that lost the *Sh* and *Bz* loci as a consequence of the nonmedian position of breakage in the first-anaphase bridge configuration. Variegation for *Wx* and *wx* within this sector is brought about by the subsequent breakage-fusion-bridge cycle that eliminates the *Wx* locus from some nuclei. The sister sector will show a *C Sh Bz Wx* phenotype; but within this sector subsectors will be present. Their constitutions will be *C sh Bz Wx, C sh bz Wx,* or *C sh bz wx*. These subsectors arise from losses of *Sh, Bz,* and *Wx* loci from some of the cells as a consequence of the breakage-fusion-bridge cycle initiated by the original *Ds* event. The variegation pattern appearing

in the kernels with *Ds* located between *I* and *Sh* is thus in striking contrast with that produced in kernels having *Ds* at its standard location. Other locations of *Ds* activity in the short arm of chromosome 9 are likewise readily detected by the type of phenotypic characters expressed in the sectors of the exceptional kernels. These exceptional kernels, therefore, make it possible to identify immediately those kernels that carry newly arising transposed *Ds* loci. From such exceptional kernels have been derived the strains with changed locations of *Ds* activity. A study of these strains has provided the information needed to interpret the mechanism responsible for transposition.

Many of the exceptional kernels appearing in the cross described above are defective in growth of either the endosperm or the embryo. They do not germinate. Other exceptional kernels appear to be morphologically normal in both endosperm and embryo, but many of them also do not germinate. Less than half the selected exceptional kernels have given rise to viable plants. The plants obtained from the viable fraction, however, have been subjected to extensive cytological and genetical tests to determine the exact location of the new positions of *Ds* activity in each case. These tests have revealed that *Ds* may be inserted at various positions within the short arm of chromosome 9. Its insertion adjacent to the *C* locus, which gave rise to the mutable c^{m-1}, has been described previously. Recently, a mutable *bz* locus appeared in an exceptional kernel; and a plant was obtained from this kernel. Preliminary tests suggest that the behavior of this mutable *bz* locus may be similar to that observed for c^{m-1}. It is now being investigated to determine whether or not it arose by insertion of *Ds* adjacent to the *Bz* locus.

Events Occurring at the *Ds* Locus

Considerable progress has been made during the year in understanding the nature of the events that occur at the *Ds* locus in somatic cells. It is believed that only one primary type of event occurs at this locus, and that it has several different types of consequences. These consequences resemble those produced by treating cells with X-rays, ultraviolet rays, chemicals, and so forth, in that they involve some mechanism leading to chromosome breakage and fusion. The several types of chromosomal alteration that are repeatedly observed when *Ds* is present can be readily understood if it is assumed that the primary event produces a physical change of the material composing *Ds:* that this material becomes sticky or adhesive in certain cells at predetermined times in the development of a tissue. The known consequences of events occurring at *Ds* will illustrate the reasons for the assumption. They may be enumerated: (1) Dicentric chromatid formation, with fusion of sister chromatids at the position of *Ds*. This is accompanied by formation of an acentric fragment composed of the two sister-chromatid segments, from *Ds* to the end of the arm. (2) Deletion of chromatid segments of various lengths adjacent to *Ds*, usually with concomitant loss of *Ds* activity but occasionally without loss of this activity. (3) Loss of detectable *Ds* activity without visible alteration of the chromosome. (4) Reciprocal translocations involving chromosome 9, in which one breakage point is at *Ds*. (5) Duplication of segments of chromosome 9, with one break marked by the known position of the *Ds* locus. (6) Transposition of *Ds* activity from one position to another in the chromosome complement, with or without an associated gross chromosomal rearrangement. (7) Composition changes

at the *Ds* locus that result in precise changes in the relative frequencies of occurrence in future cell generations of the various events enumerated above.

In previous reports, this last occurrence has been termed "change in state" of *Ds*. A state giving a high frequency of event 1 and relatively high frequencies of events 2, 4, and 5 sometimes changes, after a somatically occurring event at *Ds*, to a state giving low frequencies of these events and a high frequency of event 3. A return from this latter state to the former state requires a progressive series of events at *Ds*. This was determined in the following manner. The frequency of the various types of events at *Ds* may be deduced by observing the type and pattern of variegation on the F_1 ear in crosses of *Ds*-carrying plants to tester plants. If the *Ds* locus gives a high frequency of event 1, the majority of the kernels on the test ears will express this frequency in a similar manner. There may be a few exceptional kernels, however, that show a greatly reduced frequency of event 1. The latter kernels are selected and plants are grown from them. These plants also are crossed to tester plants, to observe the action of *Ds* in the kernels on the resulting ears. With very few exceptions, the action of *Ds* in these kernels is quite similar to its action in the kernel that gave rise to the plant carrying the modified *Ds*. Occasionally, however, a kernel is observed that shows a slightly higher rate of event 1. This kernel is selected from the ear, a plant is grown from it, and a similar test is made for the action of this selected *Ds* locus. Again, the action of *Ds* in the majority of the kernels on the test ear is similar to that in the kernel that gave rise to the plant. A few kernels, however, may show an increased rate of event 1. These are in turn selected, plants are grown from them, and the test of *Ds* action is continued. In the experiments so far conducted, it has required three or four generations of such progressive selection to obtain plants carrying a *Ds* locus giving a frequency of event 1 that resembles the frequency given by the original *Ds* from which it was derived.

The Mechanism of Transposition of *Ds*

An interpretation of the mechanism of transposition of *Ds* was considered in Year Book No. 48. It was based on analysis of those transpositions of *Ds* that were accompanied by gross chromosomal rearrangements. During the past year, two such cases have been extensively analyzed. The nature of the alteration, in each case, with respect to chromosomal constitution and genic arrangement was determined with considerable accuracy. In each case, a duplication of a segment of the short arm of chromosome 9 accompanied the appearance of a new location of *Ds* activity. Each case arose from a different male parent, and was originally identified because of an exceptional variegation pattern appearing in a single kernel on an ear produced by crossing a *C sh bz wx* tester plant by an *Ac*-carrying plant that had two morphologically normal chromosomes 9—one with the markers *I, Sh, Bz, Wx,* and *Ds*-standard, and the homologue with the markers *C, sh, bz,* and *wx,* and no *Ds* locus. In each case, the duplication arose from breaks at comparable positions in sister chromatids of the *I Sh Bz Wx Ds*-standard chromosome. Likewise, in each case, one break occurred at the *Ds*-standard position, the second break at the position marked by the new location of *Ds*, that is, just to the right of the *I* locus in one case, and a short distance to the left of the *I* locus in the other. The duplicated segment in the first case was

in the inverted order, in the second case in tandem order. Diagrams illustrating the chromosome composition and genic order in the two cases are given in figure 1.

As the diagrams show, two *Ds* loci are present in each chromosome. Their positions in each case mark the breakage points in the sister chromatids of the *I Sh Bz Wx Ds*-standard chromosomes that gave rise to the duplication. In the case illustrated in figure 1*a*, no *Ds* locus is present at the standard position in the distal segment, although a break in the chromatid occurred at this location. This position marks the proximal breakage point of the inverted duplicated segment. In the case illustrated in *b*, no *Ds* locus is present at the standard location in the proximal duplicated segment, even though it is obvious that a break occurred at this position to give rise to the chromosome with this particular duplication. If the primary event occurring at a *Ds* locus is related to a physical change causing adhesiveness in the material composing *Ds*, then the origin of these duplications, with the described breakage points, genic organization, and altered positions of *Ds* activity, can be understood. According to this assumption, the *Ds* material in the *I Sh Bz Wx Ds*-standard chromosome adhered to another position in the short arm of chromosome 9—immediately to the right of *I* in the first case, and to the left of *I* in the second case. Stresses produced by chromosome movement during the mitotic cycle that followed resulted in rupture of the sister chromatids at the adhered regions. Subsequent fusions of broken ends produced the duplications with the altered positions of *Ds*.

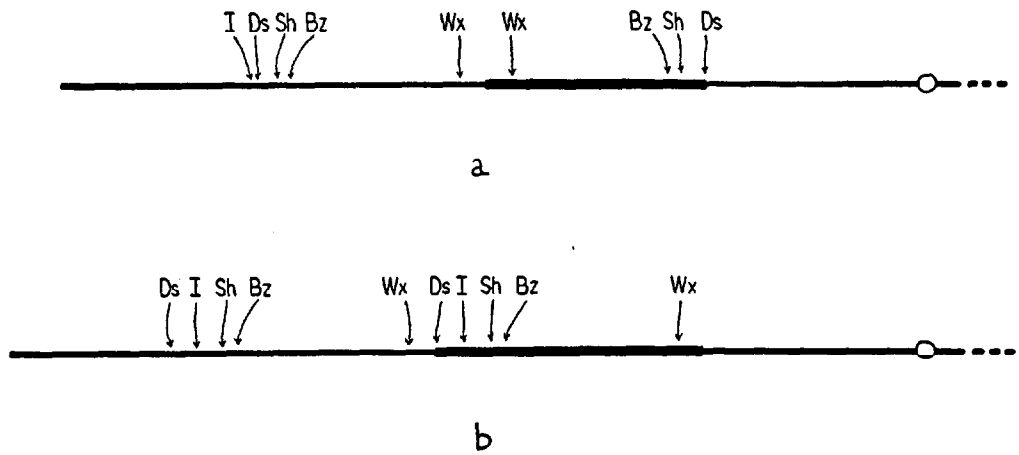

FIG. 1. Diagrams *a* and *b* illustrate two different duplications of segments of the short arm of chromosome 9. The wide line indicates the duplicated segment in each case; the open circle represents the position of the centromere. The arrows point to the locations of the genetic markers carried by each of these chromosomes.

Various types of chromosomal rearrangement, with or without accompanying transpositions of *Ds*, could result from the above-described mechanism. Figure 1 illustrates only two such cases. It is readily apparent that in these cases the fusions could have given rise to morphologically normal chromosomes 9, each carrying a transposed *Ds* locus but showing no other alteration in genic organization. The types of fusion that followed the breakages

would determine the consequences. Therefore various other kinds of gross chromosomal rearrangements, with one breakage point at the known location of *Ds*, could be anticipated. Just such rearrangements have frequently been observed in the sporocytes of plants carrying *Ds* at known positions in chromosome 9. The majority of viable exceptional kernels carrying newly arisen transpositions of *Ds*, however, have not produced plants with such gross chromosomal rearrangements. Most often, a chromosome 9 with a transposed *Ds* locus is morphologically normal. This was to be expected, for the kernels having newly arisen transpositions of *Ds* were selected from ears produced from crosses in which the *Ds* locus was brought in by the pollen parent. The pollen grains with unbalanced chromosomal complements do not compete favorably with those having a normal chromosome complement. Consequently, many of the pollen grains carrying transpositions of *Ds* associated with gross chromosomal rearrangements may be expected not to function. It is reasonable to believe, therefore, that the above-described cases having a transposition of *Ds* associated with a gross chromosomal rearrangement are representative examples, illustrating the mode of transposition of *Ds*.

As stated earlier, the transposition mechanism introduces *Ds* at various positions in the short arm of chromosome 9. Apparently, it may enter any position within this arm. The analysis of c^{m-1} indicated that the insertion of *Ds* adjacent to the *C* locus brought about an inhibition of the *C* action, which was withdrawn when *Ds* was subsequently removed from this position. If *Ds* inhibits the action of adjacent genes, then newly arising *Ac*-controlled mutable loci should continue to appear as the result of continued transpositions of *Ds* to new locations. Detection of such mutability depends on experimental procedures. Crosses of plants carrying known recessive markers in chromosome 9 by plants having the dominant alleles and also *Ds* can reveal the presence of mutability of one of the marked loci directly on the F_1 ears. Sectors within a kernel that show newly arising mutability for the marked loci are not uncommon after such crosses. In order that a strain having such a newly arising mutable locus may readily be obtained, it is necessary that the causative event occur before the two sperm nuclei are formed, so that both the endosperm and the embryo will carry the mutable locus. Since *Ds* events may occur in only a few cells, late in the development of the sporogenous tissues, relatively few kernels can be anticipated that will show new germinal origins of mutability for the marked loci. The few exceptional kernels that do show such mutability may be selected from the ear for continued study.

Newly arising mutability for previously unmarked loci may be detected in the progenies derived from self-pollinations. Ease of detection requires a sharp delineation of contrasting phenotypic characters capable of being expressed in small sectors. Morphological determiners, or physiological determiners requiring special techniques for detection, are not suitable for such a study, even though they may constitute the largest group of mutable loci. In the culture under investigation, many new recessive mutations effecting marked changes in morphological characters have appeared in the progeny of self-pollinated plants. It is not practicable to make a precise study to determine whether or not mutability is being expressed at each of these recessive loci, even though the mutable behavior of a few of them has been obvious.

Transposition and Change in Action of *Ac*

The transposition of the *Ac* locus from one position in the chromosome complement to another, frequently from one chromosome to another, was described in Year Book No. 48. The relation between loss and transposition of *Ac* was likewise described, along with the methods used to detect the kernels that show loss or transposition. During the past year, this study was greatly expanded. All together, 69 independent cases of loss, transposition, or change in action of *Ac* were investigated. For 58 of these cases, the method of analysis was similar to that described in Year Book No. 48, and therefore only the results obtained need be considered here.

In 42 cases, kernels were selected because they showed no *Ds* activity in the cross of *Ac Ac* (allelic positions) by *Ds ac* tester plants. To determine why *Ds* activity was absent, plants were grown from these kernels and tests conducted for the presence of *Ac*. In 19 plants, no *Ac* locus was present. In 17 plants, two independently inherited *Ac* loci were present, indicating that a transposition of *Ac* had occurred in a cell of the female parent of these plants. In 6 plants, there was present an *Ac* locus that was inherited as a single unit. Its action, however, resembled that of two doses of the *Ac* locus from which it was derived. The reasons why *Ds* activity did not appear in these 42 selected kernels have been presented previously. They are related in 19 of the cases to the absence of *Ac*, and in the other 23 cases to the high dose of *Ac* present in the endosperms, which so delayed the occurrence of *Ds* events that none was observed.

Eight other kernels were selected from the ears of the mentioned cross because they showed a very obvious delay in the time of occurrence of *Ds* events. Only a few very late-occurring *Ds* events were evident in these kernels. Analysis of the *Ac* constitution of the plants derived from them showed that in 5 cases two non-linked *Ac* loci were present. In 3 cases, an *Ac* locus inherited as a single unit but giving a double-dose type of action was responsible for the delay in the timing of *Ds* events.

In 8 more aberrant kernels selected from the ears of this cross, the dosage-type action of *Ac* appeared to have increased moderately, although not to an extent resembling the double-dose action in the above-mentioned cases. The analysis of *Ac* action in 7 of the 8 plants derived from these kernels indicated that the cause for the altered timing of *Ds* events was related to an alteration of *Ac* that had occurred in a cell of the parent plant late in its development. In the eighth case, the *Ac* action resembled that given by the *Ac* loci in the parent plant; no obvious change in action was detected.

Tests of *Ac* action in 21 plants derived from kernels that showed no alteration in *Ac* action in the above-mentioned cross served as a control. In all 21 cases it was obvious that no detectable change in *Ac* action had occurred in the ancestor cells of the *Ac Ac* plant that gave rise to these kernels. With respect to events that occur at the *Ac* loci in some of the *Ac Ac* (allelic positions) plants, resulting in transposition, change in action, or loss of *Ac* from the gametic nuclei, the above findings are similar to those previously described.

Seven independent cases of transposition of *Ac* from an unknown location in the chromosome complement to chromosome 9 have been detected. In 4 of these cases, *Ac* was transposed to the long arm of chromosome 9; in 2 cases, to the short arm of chromosome 9. In 1 case, the exact

location of *Ac* remains to be determined. The presence of an *Ac* locus in the short arm of chromosome 9 is most strategic for determining the events that occur at *Ac,* because of the excellent group of genetic markers carried by this arm. One such case has received sufficient study to permit some definite conclusions regarding events that occur at *Ac*. Plants were obtained that carried *Ac* in one chromosome 9 and no *Ac* in the homologous chromosome 9. The pachytene configurations in some of these plants were examined. It was possible to detect in some cells an alteration in one chromosome 9 at a position slightly proximal to the middle of the arm. At this position, one chromosome showed a deep-staining chromomere not present in the homologue. Sometimes a tiny loop was present at this position in one chromosome. Linkage experiments, utilizing markers in the short arm of chromosome 9, place this *Ac* locus between *Bz* and *Wx,* at a position approximately four crossover units distal to the *Wx* locus. This position accords well with the position of the observed alteration in chromosome 9. Although there is not yet enough evidence to state definitely that the described alteration in chromosome 9 is at the site of the *Ac* locus, it may be anticipated that a correlation will be found. Transposition of this *Ac* locus to another chromosome of the complement, and absence of this locus in a few gametes produced by plants that are homozygous for it, have been observed.

It has been emphasized that *Ds* and *Ac* are alike in that both undergo the transposition phenomenon. Breakage events will occur at *Ds* only when *Ac* is present. It cannot yet be stated that the reciprocal is true—that is, that *Ds* must be present for *Ac* to act. This may be difficult to determine with the required degree of accuracy in the material now available. Events occurring at *Ds* produce chromosome breaks, as previously described. That the transposition of *Ds* is a reflection of this type of event has been emphasized. Is the transposition of *Ac* brought about by a similar mechanism? In general, are the events occurring at *Ac* similar to those occurring at *Ds?* Preliminary study of chromosome events at the location of *Ac* in the short arm of chromosome 9 has shown that breaks occur at this locus in somatic cells. In this respect the events at *Ac* very much resemble those at *Ds*.

Consideration of the Chromosome Materials Responsible for the Origin and Behavior of Mutable Loci

Because the same types of mutability as those observed in maize have been described for a wide variety of organisms, it is probable that the same events, involving the same chromosome materials, may occur in all organisms. Heterochromatin is suspected to be the nuclear material primarily responsible for the origin and behavior of mutable loci. It has been observed in many organisms; it appears to be present in all those examined, and occupies rather precise positions in the chromosome and nucleus. Its apparently universal presence indicates that it must have a special function in the nucleus. Since it is suspected that one phase of the function of heterochromatin may be revealed by the behavior of mutable loci, it is necessary to explain why heterochromatin is believed to be associated with mutability. Only the more pertinent evidence pointing to this relationship will be considered here.

In the present study, the original burst of newly arising mutable loci appeared in plants whose chromosome-9 short arm had

been subjected to drastic structural alterations as the consequence of a number of successive breakage-fusion-bridge cycles occurring in early developmental periods. A cytological study was made of the chromosome constitution in a number of these plants, in order to determine what types of structural change had occurred. In addition to the expected types of alteration of the chromosome-9 short arm, a number of quite unexpected types of chromosomal aberration appeared in many of the plants. With few exceptions, the heterochromatic materials—that is, the centromeres, the knobs, and the nucleolus organizer—had been involved in events leading to the unexpected chromosomal aberrations. In most cases the structural alteration arose from fusion of the knob or the centromere of chromosome 9 with other knobs or centromeres, or with the nucleolus organizer. Unquestionably, the somatically occurring breakage-fusion-bridge cycles involving chromosome 9 were responsible for the origin of these unusual types of structural alteration in the chromosome complement.

To assume from the above observations alone that alterations in heterochromatin are somehow responsible for the origin of new mutable loci would not be defensible. When considered with reference to other evidence pointing in the same direction, however, the correlation becomes more apparent. Some of this additional evidence will be considered. A case in maize analogous to the *Ac*-c^{m-1} relationship has been investigated by Dr. M. M. Rhoades. The activator in this case is designated *Dt* (Dotted), and in the strains used was found to be located in chromosome 9. It causes a recessive locus in chromosome 3, a_1 (colorless aleurone), to mutate to dominant alleles of the A_1 phenotype. Just as c^{m-1} does not mutate unless *Ac* is present in the nucleus, neither will a_1 mutate unless *Dt* is present. Tests have shown that *Ac* does not substitute for the known *Dt* locus; the two appear to be different controllers of mutable events. The similarities between the two cases are striking, and a common type of causative factor undoubtedly exists. That a heterochromatic element is involved has been suggested by the genetic studies of Rhoades, which have placed the examined *Dt* locus in the heterochromatic knob terminating the short arm of chromosome 9. The following questions arise: Is *Dt* action a reflection of some specific alteration in this heterochromatic material? Could this alteration be produced anew? It was thought that since the breakage-fusion-bridge cycle gives rise to various alterations in heterochromatic elements, it might be possible, by subjecting tissue to this cycle, to re-create the *Dt* effect.

The experimental procedure aimed at re-creating the *Dt* effect has been as follows. The plants used as the female parents had normal chromosome constitutions, were homozygous for a_1, and carried no *Dt* locus. Extensive tests by several investigators have shown that no mutations of a_1 to A_1 will occur when such plants are crossed by plants of similar chromosomal and genic constitutions. In this experiment, however, these plants were crossed by plants that were homozygous for a_1 and carried no *Dt* locus, but whose chromosome constitution was not normal. In these, one chromosome 9 was deficient for a long terminal segment of the short arm; the homologous chromosome 9 carried an inverted duplication of the short arm. More than half of the functioning pollen grains of such plants carry a chromosome 9 with a newly broken end; and breakage-fusion-bridge cycles occur in the endosperms of kernels receiving such broken chromosomes. If

the *Dt* effect is due to a specific structural alteration in the heterochromatin, then this particular alteration might be re-created in some of the kernels as a consequence of this cycle. Should this occur, the newly arising *Dt*-type effect would be evident in the appearance of spots of the A_1 phenotype in some of the kernels resulting from this specifically constructed cross. The kernels on 95 ears resulting from this cross were examined. Most of them were colorless, but a total of 15, coming from 14 different ears, had typical A_1 spots. Nine of these kernels had a single A_1 spot, three had 2 A_1 spots, two had 4 A_1 spots, and one had 5 A_1 spots. The A_1 spotting on these kernels was similar to that produced by the known *Dt* locus. These preliminary results are in accordance with expectation. The experiment must be continued, however, to determine whether or not the mutations arise from alterations in heterochromatic elements, as required by the basic assumptions.

It is in *Drosophila melanogaster* that the greatest body of evidence bearing on the relation of heterochromatin to genic instability has been accumulated. The "position effect" variegations in this organism have been extensively investigated. Heterochromatin components are known to control the type and time of somatically occurring changes in genic action. Comparisons of the behavior of the various types of heterochromatically controlled "position effect" variegations in *Drosophila* with the behavior of various types of mutable loci in maize have shown a marked similarity in the kinds of variegation expression in these two organisms. Even the dosage action of the heterochromatic Y chromosome in *Drosophila* and that of *Ac* in maize have resemblances that may not be coincidental. These similarities make it necessary to consider a common causative mechanism to account for the occurrence of the same types of phenotypic expression in the two organisms, especially since the evidence in maize likewise points toward heterochromatic control. The converging evidence from the two organisms may provide the supplemental information necessary for a better understanding of the normal function and behavior of heterochromatin.

That an interrelation exists between the heterochromatic elements of the chromosomes and the gene-carrying euchromatic elements can hardly be questioned. The apparently universal presence of heterochromatic components of the chromosome complement, the particular spatial positions that they occupy in these chromosomes and in the working nucleus, the phenotypic effects produced by altering the quantity, organization, or position of heterochromatic elements with respect to euchromatic elements, and the absence or infrequent occurrence of phenotypically recognized Mendelizing units within the heterochromatin, all suggest a functional property of heterochromatin quite different from that expressed by the euchromatic elements of the chromosome complement. These interrelations and differences make it necessary to intensify efforts to clarify the functional aspects of heterochromatin in the working nucleus. This must be done before it will be possible to interpret adequately the mode of operation of the euchromatic elements that carry the Mendelizing units. If further evidence substantiates the present indication that changes in heterochromatic elements are responsible for the origin and behavior of mutable loci, then all the observations made concerning the behavior of these loci will be relevant in unraveling the functional aspects of heterochromatin, and some of the details of the mode of operation of heterochromatic elements will be made evident.